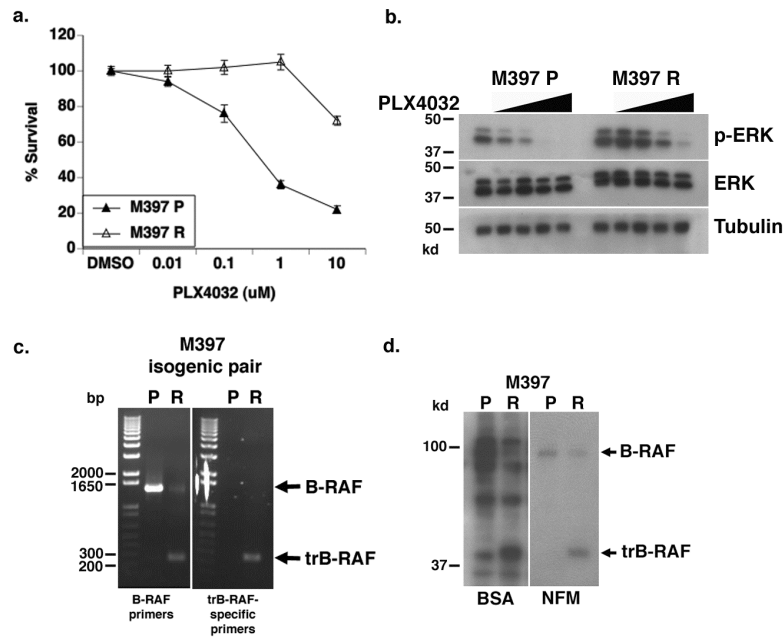
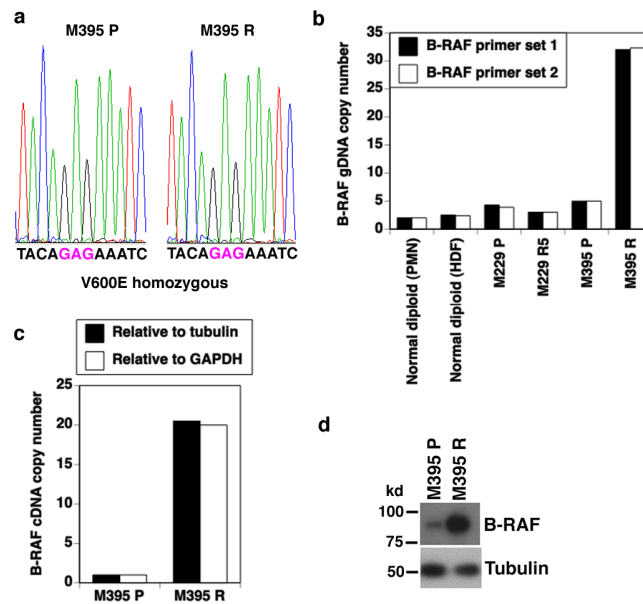


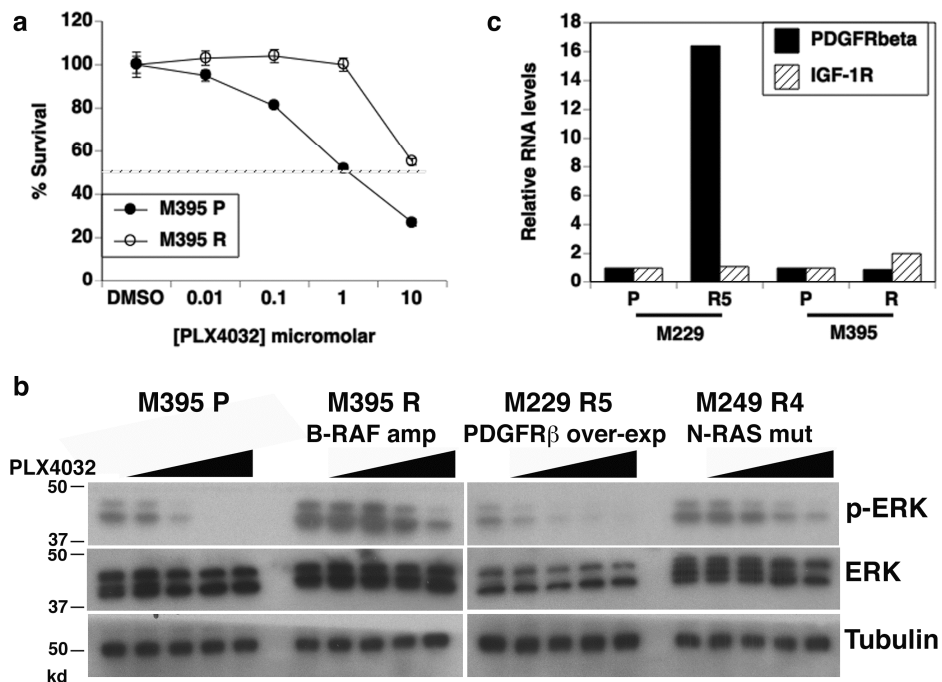
Supplementary Figure S1. RTK and COT expression levels in patient-matched melanoma tissues. Paired RNAs/cDNAs from baseline (B) and disease progression (DP) melanomas (from patients treated with either vemurafenib or dabrafenib) were subjected to Q-PCR, measuring the levels of indicated transcripts. Results are the average of duplicates. Patient numbers correspond to those in Supplementary Table S1.



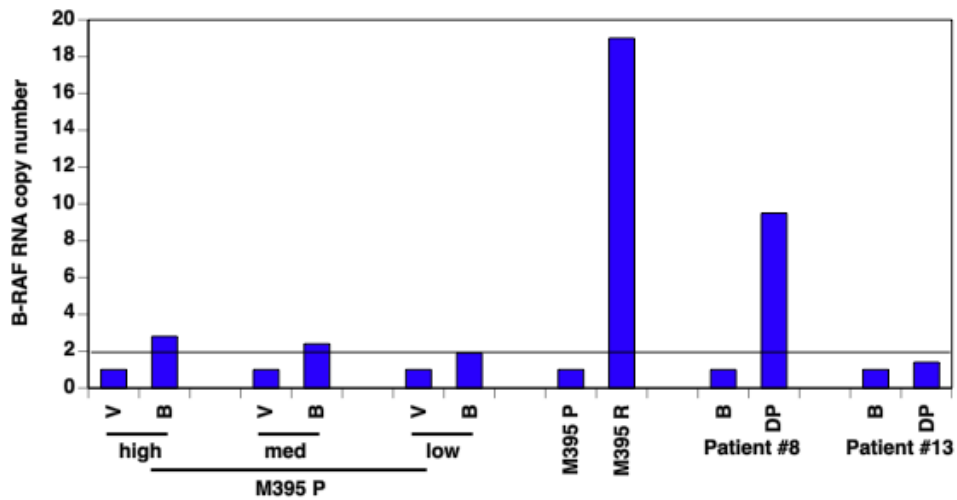
Supplementary Figure S2. A vemurafenib-resistant, ^{V600E}B-RAF melanoma cell line displaying ERK reactivation and harboring trB-RAF. (a) M397 parental (P) cell line was treated with incremental concentrations of PLX4032/vemurafenib over four weeks, deriving a resistant (R) sub-line, M397 R. Isogenic lines were treated with increasing concentrations of vemurafenib/PLX4032 or DMSO (vehicle), and cells quantified by a MTT assay. Survival curves are shown after 72 h drug treatments, and data represent percent surviving cells relative to DMSO-treated controls (mean \pm SEM, n = 5). (b) Isogenic paired cell lines were treated with PLX4032 at 0, 0.01, 0.1, 1.0 and 10 μ M of PLX4032 (1 h) after 24 h seeding period during which M397 R was withdrawn from routine maintenance treatment with 1 μ M PLX4032. (c) cDNAs from M397 parental (P) and vemurafenib-resistant (R) cell lines were subjected to PCR to detect full-length B-RAF and the B-RAF variant (truncated B-RAF) (left) and to detect trBRAF specifically using an exon1-exon11 junction primer (right). (d) Protein lysates from M397 P and M397 R were probed by western blotting for the presence of both full-length B-RAF (90 KD) and trB-RAF (40 KD) using a B-RAF antibody raised against the C-terminal end. Blocking using bovine serum albumin (BSA) vs. non-fat milk (NFM).



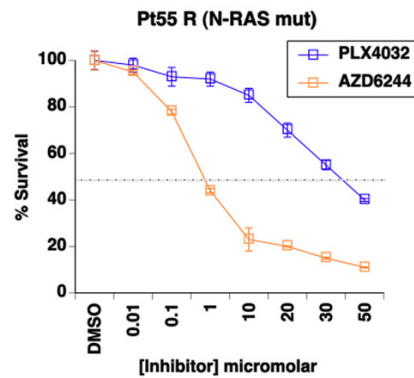
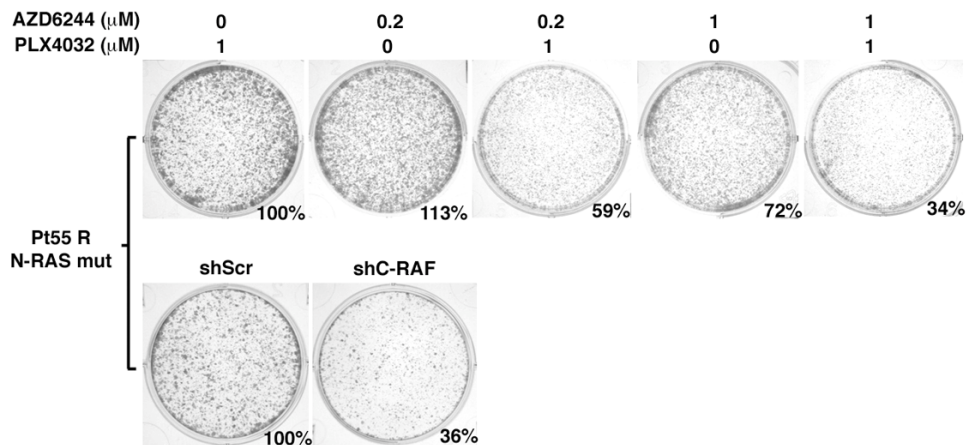
Supplementary Figure S3. Amplification of $V600E$ B-RAF in the M395 isogenic model of acquired B-RAFⁱ resistance. (a) M395 was established from a non-regressing adrenal metastasis in a patient treated with vemurafenib after 5 months on therapy. The cell line established from the tumor biopsy was not cultured in vemurafenib, and this cell line maintained *in vitro* sensitivity to vemurafenib and was designated as M395 P. The resistant sub-line, M395 R, was derived by titrating increasing concentration of PLX4032 from 0.01 to 10 μ M over two months. Both P and R lines are V600E homozygous at *B-RAF*. (b) Copy numbers of $V600E$ *B-RAF* as determined by gDNA Q-PCR (normalized to *globin* levels) and relative to diploid cells (PMN and HDF) using two independent sets of primers. Results are average values of duplicates. (c) $V600E$ *B-RAF* levels as determined from RNA/cDNAs using Q-PCR (normalized to either *tubulin* or *GAPDH*). (d) $V600E$ B-RAF protein levels as shown by western blotting (tubulin, loading control).



Supplementary Figure S4. RTK-independent, ERK reactivation in the M395 vemurafenib-resistant sub-line. **(a)** Survival curves of an isogenic cell line pair to 72 h of PLX4032 treatments, suggesting a drug-saturable resistance mechanism in M395 R. Results are shown relative to DMSO-treated controls (mean \pm SEM, $n = 5$; dashed line, 50% inhibition). **(b)** Q-PCR levels of indicated RTKs using RNA/cDNA for two isogenic models of PLX4032 acquired resistance. M229 R5 is known to over-express PDGFR β . Results are average values of duplicates (relative to tubulin). **(c)** pERK level modulation by PLX4032 (0, 0.01, 0.1, 1, and 10 μ M) in PLX4032-resistant lines with different mechanisms, suggesting MAPK reactivation in M395 R as in M249 R4.



Supplementary Figure S5. Relative *B-RAF* RNA copy numbers in cell lines and tissues. M395 P was transduced stably with an empty lentivirus (V for empty Vector) or a lentivirus carrying ^{V600E}*B-RAF* (B) (corresponding ^{V600E}*B-RAF* protein levels shown in Figure 2a) at various titers. *B-RAF* RNA copy numbers for these stable cell lines were then determined along with (for comparison) those for M395 P vs. M395 R isogenic cell lines as well as for two sets of patient-matched baseline (B) and disease progression (DP) tissues. *B-RAF* levels were determined from RNA/cDNAs using Q-PCR (normalized to tubulin). Results are average values of duplicates. Line indicates a two-fold change.

a**b**

Supplementary Figure S6. A mutant N-RAS-driven, vemurafenib-resistant short-term melanoma culture and its sensitivity to B-RAF_i, MEK_i, their combination and C-RAF

knockdown. **(a)** Survival curves of Pt55 R1 to 72 h of PLX4032 (vemurafenib/B-RAF_i) or AZD6244 (selumetinib/MEK_i) treatments, showcasing differential responses at the micro-molar drug concentration range. Results are shown relative to DMSO-treated controls (mean ± SEM, n = 5; dashed line, 50% inhibition). **(b)** Clonogenic assays of using Pt55 R1 with indicated drug treatments (top, 12 days) or stable C-RAF knockdown (bottom, 10 days, with 1 μM PLX4032). Inhibitors and media were replenished every two days, colonies visualized by crystal violet staining, and quantified (% growth relative to cells treated with 1 μM PLX4032 (top) or shScr (bottom)). Photographs representative of two independent experiments.

Supplementary Table S1. Biopsy sites of patients studied.

Study Site	Pt #	Biopsies	Anatomic Bx Sites
UCLA	1	B	Lymph node- femoral
		DP1	Lymph node- inguinal
		DP2	Small bowel
		DP3	SC and cutaneous- L groin
	2	B	SC- shoulder
		DP	Heart
	3	B	Lymph node- R axillary
		DP	Soft tissue- abdomen
	4	B	SC- L base of neck
		DP1	SC- L neck
		DP2	SC- L base of neck
		DP3	SC- L shoulder
	5	B	Lymph node- L inguinal
		DP1	Cutaneous- L ant thigh, superior
		DP2	Cutaneous- L ant thigh, inferior
	6	B	Lung
		DP	Pelvic
	7	B	SC- L lower flank/buttock
		DP1	SC- L lower flank/buttock
		DP2	Soft tissue- L breast
	8	B	SC- scalp
		DP	SC- R chest
	9	B	SC- abdomen
		DP1	SC- R chest
		DP2	Cutaneous- L shoulder
	10	B	Cutaneous- L leg
		DP1	Cutaneous- L foot
		DP2	Cutaneous- L leg, medial
		DP3	Cutaneous- L leg, lateral
	11	B	SC- R axillary
		DP	SC- back
	12	B	SC- abdomen
		DP	SC- R flank
	13	B	Soft tissue- pelvis
		DP	Soft tissue- pelvis
MIA	14	B	SC- L chest
		DP	SC- abdomen
	15	B	SC- Upper chest
		DP	SC- abdomen
	16	B	Lymph node- R inguinal
		DP	Brain
	17	B	Lymph node- R neck
		DP	SC- R neck
	18	B	SC- L groin
		DP	SC- L flank
VI	19 Pt56	B	Lymph node- inguinal
		DP	Soft tissue- pelvis
	20 SL	B	SC- R neck
		DP	SC-R leg

Supplementary Table S2. Exome sequencing data characteristics.

Pt #8			
	Normal	Baseline	DP
Library	50+50 PE, 100+100 PE	50+50 PE, 100+100 PE	50+50 PE, 100+100 PE
Total read count	198,535,632	270,137,370	256,439,396
Capture specificity	43.2%	44.1%	42.3%
% of targeted base covered at $\geq 10\times$	89.5%	90.3%	90.6%
Average Coverage	107.6 x	132.6 x	123.3 x

Type of somatic alterations	DP-specific #
Non-synonymous or nsSNVs	4
INDELs	0
CNVs	871 (468:Amplified, 403:Deleted)

Pt #5			
	Normal	Baseline	DP
Library	76 SE	76+76 PE	76+76 PE
Total read count	62,448,536	137,656,936	147,415,956
Capture specificity	75.2%	78.1%	74.7%
% of targeted base covered at $\geq 10\times$	88%	92%	93%
Average Coverage	52.7 x	88.8 x	114.3 x

Type of somatic alterations	DP-specific #
Non-synonymous or nsSNVs	1
INDELs	0
CNVs	734 (424:Amplified, 310:Deleted)

Supplementary Table S3. DP-specific somatic nsSNVs.

Pt #8											
Chr.	Position	R e f	V a r	P value (DP vs. normal)	P value (DP vs. baseline)	Accession ID	Gene	AA change	AA position	PhyloP Score	Polyphen
4	8609063	C	T	1.25E-004	4.28E-005	NM_001014447,N M_001014448,N M_003652	CPZ	HIS/TYR	380/653,243/ 516,369/642	5.362	probably damaging
4	101108952	A	T	3.23E-013	5.26E-017	NM_145244	DDIT4L	PHE/TYR	155/194	2.674	benign
4	109745350	G	C	1.53E-013	8.87E-017	NM_032518,NM_ 198721	COL25A1	LEU/VAL	609/643,609/ 655	-0.041	benign
4	110791146	C	T	1.32E-022	1.62E-034	NM_198506	LRIT3	PRO/LEU	369/635	2.898	possibly damaging
8	95839582	G	C	8.18E-023	5.33E-032	NM_017864	INTS8	ALA/PRO	133/996	0.907	benign
8	124792307	T	C	6.02E-012	1.90E-020	NM_144963	FAM91A1	VAL/ALA	211/839	3.5	benign
8	134125756	C	T	2.01E-009	6.69E-018	NM_003235	TG	ARG/CYS	2555/2769	-1.964	probably damaging
10	11894129	C	T	1.59E-005	3.89E-006	NM_153256	C10orf47	SER/PH E	18/436	1.599	probably damaging
10	13225081	C	T	2.07E-025	2.07E-025	NM_018518,NM_ 182751	MCM10	PRO/LEU	360/875,361/ 876	3.903	possibly damaging
10	17641343	G	A	1.22E-014	2.08E-015	NM_014241	PTPLA	SER/PH E	184/289	6.163	possibly damaging
10	19856502	G	A	4.96E-025	4.15E-025	XM_295865	C10orf112	TRP/sto p	1560/1818	4.889	
10	45878069	G	A	2.21E-007	1.95E-008	NM_000698	ALOX5	GLU/LYS	97/675	5.36	probably damaging
10	79769683	G	A	2.58E-017	1.05E-014	NM_007055	POLR3A	SER/LEU	570/1391	5.708	benign
10	91520377	C	T	4.06E-016	3.43E-015	NM_016195	KIF20B	SER/PH E	1552/1781	2.821	possibly damaging
10	106982927	G	A	8.71E-040	1.25E-051	NM_014978	SORCS3	GLU/LYS	930/1223	5.526	possibly damaging
10	131665425	G	T	0.0225472	0.0336512	NM_001005463	EBF3	PRO/HIS	331/552	5.984	probably damaging
13	113825980	C	T	7.00E-005	2.29E-007	NM_003891	PROZ	ALA/VAL	255/401	-0.203	benign
14	70512882	C	A	0.009946	0.0068087	NM_001130417,N M_033262,NM_0 58240,NM_18293 2,NM_182936,NM _183002	SLC8A3	VAL/LEU	227/299,854/ 926,853/925, 850/922,213/ 285,856/928	6.222	probably damaging
15	45393436	C	T	1.64E-007	5.54E-007	NM_014080	DUOX2	ARG/GLN	963/1549	2.902	possibly damaging
19	45296786	C	T	0.0047006	0.0276535	NM_001130852,N M_012116	CBLC	ALA/VAL	352/429,398/ 475	2.095	benign

Pt #5											
Chr.	Position	R e f	V a r	P value (DP vs. normal)	P value (DP vs. baseline)	Accession ID	Gene	AA change	AA position	PhyloP Score	Polyphen
1	184556124	C	A	1.14E-11	1.07E-19	NM_003292	TPR	ASP/TYR	2171/2364	4.96	possibly damaging
X	100418099	T	A	1.86E-4	3.55E-07	NM_024885	TAF7L	GLU/ASP	341/463	-6.19	neutral

Supplementary Table S4. Primer and shRNA sequences.

qRT-PCR	Foward	Reverse
PDGFRb	TTCCATGCCGAGTAACAGAC	CGTTGGTGATCATAGGGGAC
IGF1R	CCGCAGACACCTACAACATC	CAATGTGAAAGGCCGAAGGT
COT	CCCTGGAAGCTGACTTACA	CTGGGATCAGTTTACACGCC
B-RAF	ATGTTGAATGTGACAGCACC	CTCACACCACTGGGTAAACA
trB-RAF	TGCCATTCCGGAGGAGAAAAAC	AGGCTTGTAAGTCTGAGGTG
Tubulin	GACAGCTCTTCCACCCAGAG	TGAAGTCCTGTGCACTGGTC
GAPDH	CAATGACCCCTTCATTGACC	GACAAGCTTCCCGTTCTCAG
gDNA copy	Forward	Reverse
B-RAF set1	ACCTCAGCAGTTACAAGCCT	CACTGGGAACCAGGAGCTAA
B-RAF set2	GATATTGCACGACAGACTGCA	AGCATCCTTATGTTCTGGACA
Globin	AATTCACCCCAACAGTGCAG	CTTCCCGTTCTCAGCCTTGA
shRNA primer sequences	Sense	Antisense
shSCRAMBLED	TGGAATCTCATTGATGCATACTT CAAGAGAGTATGCATCGAATGAGATTCCTTTTTTC	TCGAGAAAAAAGGAATCTCATTCTG ATGCATACTCTCTTGAAGTATGCATCGAATGAGATTCCA
ShC-RAF1	TGACAGAGAGATTCAAGCTATTT CAAGAGAATAGCTTGAATCTCTCTGTTTTTTTC	TCGAGAAAAAAACAGAGAGATTCA AGCTATTCTCTTGAATAGCTTGAATCTCTCTGTCA
ShC-RAF3	TGCAAAGAACATCATCCATAGTT CAAGAGACTATGGATGATGTTCTTTGTTTTTTC	TCGAGAAAAAAACAAAGAACATCATC CATAGTCTCTTGAAGTATGGATGATGTTCTTTGCA
ShB-RAF1	TGACAGAGACCTCAAGAGTAATT CAAGAGATTACTCTTGAGGTCTCTGTTTTTTTC	TCGAGAAAAAAACAGAGACCTCAAG AGTAATCTCTTGAATTACTCTTGAGGTCTCTGTCA
ShB-RAF3	TGCAACAACAGGGACCAGATATT CAAGAGATATCTGGTCCCTGTTGTTGTTTTTTC	TCGAGAAAAAAACAACAACAGGGACC AGATATCTCTTGAATATCTGGTCCCTGTTGTTGCA